the ET_B gene, 2) A highly conserved region in its 5'UTR harbors an element responsible for high level expression of a reporter gene in cells expressing ET_B. These elements may help us understand ET_B regulation during neural crest development and in conditions associated with endothelial dysfunction such as heart failure, pulmonary hypertension and atherosclerosis.

1074-122 Aldosterone synthase gene polymorphism predicts left ventricular size and function in persons free of heart disease

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Aldosterone has many effects on heart and circulation. Genetic variation in aldosterone synthesis could therefore have cardiac implications. Aldosterone synthase (CYP11B2) catalyzes the last steps of aldosterone formation. We studied whether CYP11B2 gene polymorphism predicts left ventricular (LV) size, mass or function in humans.

84 persons (40 men) free of heart disease born in 1954 were studied by M-mode and Doppler echocardiography and genotyped by polymerase chain reaction for −344 cytosine/thymidine (C/T) polymorphism in the promoter of the CYP11B2 gene. The LV measurements (mean ± SD) by the C/T genotype group were as follows:

Measurement	−344TT n = 22	−344CT n = 42	−344CC n = 20	P (anova)
EDD, mm	47 ± 3	50 ± 4	51 ± 4	0.002
ESD, mm	31 ± 3	34 ± 4	35 ± 6	0.013
Mass, g	149 ± 39	169 ± 46	182 ± 58	0.082
E/A	1.7 ± 0.3	1.5 ± 0.3	1.6 ± 0.3	0.024
AFF, %	23 ± 6	27 ± 6	26 ± 6	0.004

EDD = end-diastolic diameter; ESD = end-systolic diameter; E/A = transmitral early/atrial velocity ratio; AFF = atrial filling fraction. The influence of CYP11B2 genotype on LV measurements was independent of sex, body size, blood pressure and salt intake.

We conclude that DNA polymorphism in the CYP11B2 gene predicts LV structure and function in persons free of heart disease.

1074-123 Effect of Estrogen on Protein and DNA Synthesis and Estrogen Receptor Status in Cardiac Fibroblasts

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Estrogen exerts a clinically relevant anti-atherogenic effect in women. In addition, estrogen may bind to receptors on vascular smooth muscle cells and modulate the growth of these cells. Little, however, is known about its direct effects on cardiac fibroblasts, which are responsible for extracellular matrix production in the heart. We, therefore, investigated the role of estrogen on male adult rabbit cardiac fibroblasts, which were grown until subconfluent in DMEM supplemented with 10% FBS. Western immuno slot blot analysis demonstrated that cardiac fibroblasts express estrogen receptor protein. The density of the estrogen receptor significantly increased with the degree of confluency from 24 through 120 hours in culture (685%, $p = 0.005$). Next, we investigated the effect of estrogen on DNA synthesis measuring ^{3}H-thymidine incorporation. Estrogen dose-dependently decreased DNA synthesis of cardiac fibroblasts. The maximum decrease was observed with 10 nM estrogen (78%, $p = 0.0005$). In addition, estrogen treatment decreased protein synthesis, as measured by ^{3}H-phenylalanine incorporation by 84% ($p < 0.0001$). In contrast, transforming growth factor beta 1 (TGF-β_1) protein concentrations increased by 178% ($p < 0.05$). Furthermore, treatment with 10 nM, 500 nM, and 10 μM exogenous estrogen further increased estrogen receptor protein immunoreactivity by 13% ($p < 0.0005$), 17% ($p < 0.005$), and 18% ($p < 0.005$), respectively. We, therefore, conclude that estrogens modulate protein and DNA synthesis in cardiac fibroblasts. The anti-proliferative effects of estrogen may involve the induction of TGF-β_1 and/or a positive feedback on the expression of its receptor.

1074-124 Interactions Between Estradiol and Mechanical Strain in Human Vascular Smooth Muscle Cells in Culture

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The cellular basis of the cardioprotective effects of estrogen are largely unknown. A direct inhibitory effect on vascular smooth muscle growth has been proposed, but conflicting data exist, showing stimulation of mitogenesis under some circumstances. We examined the effect of different concentrations of 17β-estradiol on mitogenesis induced by cyclic mechanical strain in human vascular smooth muscle cells in culture. Cells were grown to confluence on fibronectin-coated plates with silicone-elastomer bottoms. They were then exposed to cyclic mechanical strain (60 cycles/min, 48 h), in the presence and absence of 17β-estradiol (1 nM or 1 μM). 3H-Thymidine was measured during the last six hours. Cyclic mechanical strain induced 1.5 to 2 fold increases in DNA synthesis. While estradiol, 1 nM caused an inhibition of strain-induced mitogenesis, estradiol 1 μM caused an enhancement. Thymidine incorporation data (cpm/well) are shown below.

Control	3934 ± 388
Strain	6753 ± 388
Strain + Estradiol 1 nM	3397 ± 465
Strain + Estradiol 1 μM	8875 ± 515

The extent of inhibition of strain-induced mitogenesis by physiological concentrations of estradiol (1 nM) was attenuated by the estrogen receptor antagonist ICI 182,780 (1 nM). We conclude that estrogen, in physiological concentrations, inhibits strain-induced mitogenesis via an estrogen-receptor mediated process, and in supraphysiological concentrations, stimulates mitogenesis, probably via non-specific steroid effects. Our observations may have implications for the cardiovascular benefits of low dose estrogens and the risks associated with high dose estrogens seen in epidemiological studies.

1074-125 Proteinase Activated Receptor-2 (PAR-2)-Mediated Mitogenic Responses Are Induced By Human Mast Cell Tryptases

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PAR-2 is the second proteolytically activated receptor that mediates cell activation events by receptor cleavage. PAR-2 is expressed on vascular endothelial cells and is functionally coupled to the thrombin receptor (TR) *in vitro*. To further study previously identified mitogenic effects of PAR-2, we utilized the IL-3-dependent murine lymphoid cell line BaF3 to generate a stable cell line expressing PAR-2 (BaF3/PAR-2). Both wild-type BaF3 and BaF3/PAR-2 cells demonstrated proliferative responses when incubated with! 10% WEHI media (as the source of IL-3), as evaluated by MTT assay. In contrast, only BaF3/PAR-2 cells exhibited mitogenic responses when grown in IL-3-deficient media supplemented with PAR-2 activating peptide (SLIGRL, PAR^{39-44}). No responses were evident in BaF3/PAR-2 cells using the inactive peptide LSIGRL. *Xenopus* oocytes microinjected with PAR-2 cRNA demonstrated a dose-dependent responsiveness to the thrombin receptor activating peptide (TR^{42-47} SFLLRN), and incubation of BaF3/PAR-2 cells with 100 μM TR^{42--47} for 48 hours reproduced the proliferative response, although at ~75% of that identified using equimolar PAR^{39--44}. Because tryptase shares ~70% homology with trypsin (previously shown to activate PAR-2), we evaluated whether expressed forms of α- and β-tryptase could induce proliferative responses in BaF3/PAR-2 cells. Transient transfection of COS-1 cells with human mast cell α- and β-tryptase cDNA's demonstrated tryptase expression in supernatants and cells extracts for both forms, as evaluated by quantitative radioimmunoassay. Comparable proliferative responses were evident using conditioned media from either α- or β-tryptase expressed forms, whereas such responses were limited to α-tryptase cell extracts only. These results identify mast cell tryptases as physiological serine protease agonists for PAR-2 with implications for elucidating the molecular mechanisms regulating cell activation events mediated by proteases generated at the cell-surface during inflammatory, fibrinolytic or thrombosis-regulated pathways.